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      FILE 'MEDLINE, EMBASE, SCISEARCH, BIOSIS' ENTERED AT 10:18:09 ON 06 MAY
      2002
L1          1194 S EPF OR (EARLY PREGNANCY FACTOR?)
L2            72 S L1 (6P) (HCG OR (CHORIONIC GONADOTROPIN))
L3            35 DUP REM L2 (37 DUPLICATES REMOVED)
L4           103 S (EARLY PREGNANCY LOSS) (6P) (HCG OR (CHORIONIC GONADOTROPIN))
L5           336 S (HCG OR (CHORIONIC GONADOTROPIN)) (P) ISOFORM?
L6             0 S L4 AND L5
L7            40 DUP REM L4 (63 DUPLICATES REMOVED)

      FILE 'STNGUIDE' ENTERED AT 10:36:53 ON 06 MAY 2002

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L7 ANSWER 11 OF 40 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
IT Miscellaneous Descriptors
HCG data interpretation: reliability; **early pregnancy loss**; menstrual cycle; Meeting Abstract

L7 ANSWER 12 OF 40 MEDLINE DUPLICATE 3
AB BACKGROUND: Experience with polycystic ovary syndrome shows that insulin resistance is related to **early pregnancy loss** . This association was examined by comparing pregnancy outcome in obese and lean women. METHODS: A cohort of 383 patients conceiving. . . clomiphene citrate and FSH or hMG (n=5). Luteal phase was supported with progesterone. Pregnancies were defined by >10 IU/l plasma beta-**hCG** on day 14. Ultrasound scan on week 6 and week 12 confirmed fetal viability. RESULTS: Lean group (body mass index. . . pregnancies, or infertility diagnosis on the probability of miscarriage were not significant. CONCLUSIONS: Obesity is an independent risk factor for **early pregnancy loss**. This risk is, in part, related to the lower number of collected oocytes in obese women.

L7 ANSWER 13 OF 40 MEDLINE DUPLICATE 4
AB Human **chorionic gonadotropin** (**hCG**) is the hormone of pregnancy and forms the basis of all pregnancy tests as well as diagnostic assays for a. . . the free subunits and fragments of the hormone, especially in urine, has proven of special use for diagnosis of very **early pregnancy loss**, an important phenomenon related to infertility, as well as part of screening programs for Down Syndrome and gynecological cancers. This article summarizes existing and new methods for the preparation of **hCG**, its subunits, and the beta core fragment from urinary sources. The methods for proper analyses of these materials are also. . .

L7 ANSWER 14 OF 40 MEDLINE DUPLICATE 5
AB . . . metabolites, which changes rapidly with luteinization of the ovarian follicle. The time of implantation was defined by the appearance of **chorionic gonadotropin** in maternal urine. RESULTS: There were 199 conceptions, for 95 percent of which (189) we had sufficient data for analysis.. . . pregnancies (25 percent) ended in early loss. Among the pregnancies that lasted six weeks or more, the first appearance of **chorionic gonadotropin** occurred 6 to 12 days after ovulation; 118 women (84 percent) had implantation on day 8, 9, or 10. The risk of **early pregnancy loss** increased with later implantation (P<0.001). Among the 102 conceptuses that implanted by the ninth day, 13 percent ended in early. . . day 11. CONCLUSIONS: In most successful human pregnancies, the conceptus implants 8 to 10 days after ovulation. The risk of **early pregnancy loss** increases with later implantation.

L7 ANSWER 15 OF 40 MEDLINE DUPLICATE 6
AB There is increased risk of **early pregnancy loss** after assisted reproduction. In this study the use of serum human chorionic gonadotrophin (**HCG**) concentrations on day 12 after in-vitro fertilization (IVF) and embryo transfer was evaluated to predict pregnancy outcome. A total of 417 IVF pregnancies were included. **Early pregnancy loss** was defined as biochemical pregnancies, ectopic pregnancies and first trimester abortions. Vital pregnancies were defined as delivered singletons, multiple pregnancies and second trimester abortions. On the post embryo transfer day 12, the mean **HCG** concentration of the vital pregnancy group was significantly higher than in **early pregnancy loss** outcomes (P < 0.00001). Receiver operating characteristic (ROC) curve analysis was performed to evaluate the cut-off value of **HCG** giving maximal sensitivity and specificity in order to discriminate early pregnancy losses from vital

pregnancies. A patient with a **HCG** value higher than the calculated cut-off value (55 IU/l) had a 90% chance of having a vital pregnancy after IVF and embryo transfer. It can be concluded that a discriminatory **HCG** value on day 12 after IVF and embryo transfer cycles may be useful in predicting pregnancy outcome and may guide. . .

L7 ANSWER 16 OF 40 MEDLINE DUPLICATE 7
AB . . . 10, 12, 14, and 16 after ET for cases or the same days without ET for controls. MAIN OUTCOME MEASURE(S): beta-**HCG** was measured with a standardized microparticle enzyme immunoassay, and IVF reproductive outcome was assessed. RESULT(S): For IVF, positive implantation was. . . cycles of ET (69.6%). A total of 30 (32.6%) ended in viable pregnancies, whereas the remaining 34 (37.0%) were miscarriages. **Early pregnancy loss** accounted for 70.6% of pregnancy losses, whereas biochemical pregnancies and clinical abortions accounted for 11.8% and 17.6%, respectively. CONCLUSION(S): Our results demonstrate that patients undergoing assisted reproductive technology have an increased rate of **early pregnancy loss** compared with fertile patients. In addition, these data indicate that implantation is more frequently impaired in IVF than in oocyte donation cycles, resulting in a high incidence of **early pregnancy loss**. This suggests that implantation may be subjected to abnormal conditions in assisted reproduction.

L7 ANSWER 17 OF 40 MEDLINE DUPLICATE 8
AB **Early pregnancy loss** (EPL), detected by patterns of human chorionic gonadotrophin (**hCG**) in urine, is the biomarker employed in investigations of the impact of personal, workplace or environmental reproductive toxins on human fertility. An issue central to these studies is what, in terms of urinary **hCG** expression, constitutes an EPL. This report describes the urinary molecular forms of **hCG** expressed in menstrual cycles in which a normal pregnancy was conceived, or an EPL occurred, or no apparent conception occurred. Qualitative and significant quantitative differences in the expression of **hCG**-associated analytes were found between normal pregnancy cycles and EPL cycles. Discriminant analysis calculation based on mole fractions of the different **hCG**-associated molecules afforded 91 per cent and 80 per cent correct classification of clinical pregnancy cycles and EPL cycles, respectively. Although **hCG**-associated molecules unique to either EPL or normal pregnancy were not found, what is thought to be an early form of **hCG** is expressed both at a high frequency and at a significantly higher concentration in early normal pregnancy when compared with. . .

L7 ANSWER 18 OF 40 MEDLINE DUPLICATE 9
AB Trisomy 16, once thought to result uniformly in **early pregnancy loss**, has been detected in chorionic villus samples (CVS) from on-going pregnancies and was initially ascribed to a second, nonviable pregnancy.. . . in women under the age of 35 because of abnormal levels of maternal serum alpha-fetoprotein (MSAFP) or maternal serum human **chorionic gonadotropin** (MShCG). The other two amniocenteses were performed for advanced maternal age. Five of the 11 pregnancies resulted in liveborn infants,. . .

L7 ANSWER 19 OF 40 SCISEARCH COPYRIGHT 2002 ISI (R)
ST Author Keywords: **early pregnancy loss**; **hCG**

L7 ANSWER 20 OF 40 MEDLINE DUPLICATE 10
AB . . . been the subject of intense debate. Most, but not all, studies have reported decreased implantation and pregnancy rates and increased **early pregnancy loss** in HSPX patients. This has led to prophylactic salpingectomies prior to IVF in HSPX patients despite the lack of any. . . HSPX enlarged with ovarian stimulation,

causing fluid reflux into the uterine cavity which was only noted after human chorionic gonadotrophin (**HCG**) administration.

=>

L7 ANSWER 24 OF 40 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 97179054 MEDLINE
DOCUMENT NUMBER: 97179054 PubMed ID: 9027341
TITLE: Immunochemical mapping of gonadotropins.
AUTHOR: Berger P; Bidart J M; Delves P S; Dirnhofer S; Hoermann R; Isaacs N; Jackson A; Klonisch T; Lapthorn A; Lund T; Mann K; Roitt I; Schwarz S; Wick G
CORPORATE SOURCE: Institute for Biomedical Aging Research, Austrian Academy of Sciences, Innsbruck, Austria.. Bioage-c511@uibk.ac.at
SOURCE: MOLECULAR AND CELLULAR ENDOCRINOLOGY, (1996 Dec 20) 125 (1-2) 33-43. Ref: 53
Journal code: E69; 7500844. ISSN: 0303-7207.
PUB. COUNTRY: Ireland
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970424
Last Updated on STN: 19970424
Entered Medline: 19970417

AB As a glycoprotein hormone, human chorionic gonadotropic (**hCG**) is not a single molecular entity but this term rather comprises an array of molecular variants such as **hCG**, **hCG** beta, hCGn, **hCG** beta n, **hCG** beta cf, -CTPhCG, **hCG** beta CTP, deglyhCG, asialohCG, hCGav and the closely related molecules hLH, hLH beta and hLH beta ef. The advent of monoclonal antibodies (MCA), the availability of ultrasensitive detection systems and the recent determination of the crystal structure of **hCG**, made it possible to design special purpose diagnostic and clinical research immunoassays for **hCG**-like molecules. For more than a decade we and others have tried to refine epitope maps for **hCG** and related molecules by means of a large panel of MCA, naturally occurring metabolic variants of **hCG** (hCGn, **hCG** beta, **hCG** alpha, **hCG** beta cf, **hCG** beta CTP), homologous hormones and subunits of various species (e.g. hLH, hLH beta, hFSH, hTSH, oLH, rLH beta), chemically modified molecules (deglyhCG, asialohCG, tryptic and chymotryptic **hCG** beta and **hCG** alpha fragments) and synthetic peptides (octapeptides and longer). It appeared that all epitopes on molecular **hCG**-variants recognized by our MCA are determined by the protein backbone. Except for the two major epitopes on **hCG** beta CTP and parts of two antigenic domains on **hCG** alpha, epitopes on **hCG**-derived molecules are determined by the tertiary and quarternary structure. Operationally useful descriptive epitope maps were designed including information on assay suitability of antigenic determinants. On this basis we established ultrasensitive time-resolved fluoroimmuno-assays for **hCG**, **hCG** and hCGn, **hCG** beta and **hCG** beta n and **hCG** beta cf, **hCG** alpha and additional assays recognizing different spectra of **hCG**-variants. Such assay have been applied by us and others to the detection of pregnancy, **early pregnancy loss**, choriocarcinoma, testicular cancer, other cancers and prenatal diagnosis. However, as the molecular structure of many epitopes utilized in immunoassays of different laboratories was not resolved, comparability of results was not satisfactory. Consequently, attempts were made to compare schematic epitope maps from different research institutions. The situation has been much improved by solving the three-dimensional (3D) structure of **hCG**. It has been shown that **hCG** is a member of the structural superfamily of cystine knot growth factors like NGF, PDGF-B and TGF-beta. Each of its subunits is stabilized in its topology by three disulfide bonds forming a cystine knot. Moreover, it turned out that the disulfide bridges in their majority

have previously been wrongly assigned. Computer molecular modeling of crystallographic coordinates of **hCG** and subsequent selective combined--PCR-based and immunological--mutational analyses of **hCG** beta expressed via the transmembrane region of a MHC molecule made it possible to more precisely localize epitopes on **hCG**-derived molecules. Although the entire surface of **hCG** has to be regarded as potentially immunogenic there seems to be hot spots where epitopes are clustered in antigenic domains. These are located on the first and third loops protuding from the cystine knots of both subunits and are possibly centered around the knot itself. Ultimate answers on epitope localizations will be given by the crystal structure determination of **hCG** complexed with different Fabs.

AB As a glycoprotein hormone, human chorionic gonadotropic (**hCG**) is not a single molecular entity but this term rather comprises an array of molecular variants such as **hCG**, **hCG** beta, hCGn, **hCG** beta n, **hCG** beta cf, -CTPhCG, **hCG** beta CTP, deglyhCG, asialohCG, hCGav and the closely related molecules hLH, hLH beta and hLH beta ef. The advent of monoclonal antibodies (MCA), the availability of ultrasensitive detection systems and the recent determination of the crystal structure of **hCG**, made it possible to design special purpose diagnostic and clinical research immunoassays for **hCG**-like molecules. For more than a decade we and others have tried to refine epitope maps for **hCG** and related molecules by means of a large panel of MCA, naturally occurring metabolic variants of **hCG** (hCGn, **hCG** beta, **hCG** alpha, **hCG** beta cf, **hCG** beta CTP), homologous hormones and subunits of various species (e.g. hLH, hLH beta, hFSH, hTSH, oLH, rLH beta), chemically modified molecules (deglyhCG, asialohCG, tryptic and chymotryptic **hCG** beta and **hCG** alpha fragments) and synthetic peptides (octapeptides and longer). It appeared that all epitopes on molecular **hCG**-variants recognized by our MCA are determined by the protein backbone. Except for the two major epitopes on **hCG** beta CTP and parts of two antigenic domains on **hCG** alpha, epitopes on **hCG**-derived molecules are determined by the tertiary and quarternary structure. Operationally useful descriptive epitope maps were designed including information on assay suitability of antigenic determinants. On this basis we established ultrasensitive time-resolved fluoroimmuno-assays for **hCG**, **hCG** and hCGn, **hCG** beta and **hCG** beta n and **hCG** beta cf, **hCG** alpha and additional assays recognizing different spectra of **hCG**-variants. Such assay have been applied by us and others to the detection of pregnancy, **early pregnancy loss**, choriocarcinoma, testicular cancer, other cancers and prenatal diagnosis. However, as the molecular structure of many epitopes utilized in immunoassays of. . . schematic epitope maps from different research institutions. The situation has been much improved by solving the three-dimensional (3D) structure of **hCG**. It has been shown that **hCG** is a member of the structural superfamily of cystine knot growth factors like NGF, PDGF-B and TGF-beta. Each of its. . . out that the disulfide bridges in their majority have previously been wrongly assigned. Computer molecular modeling of crystallographic coordinates of **hCG** and subsequent selective combined--PCR-based and immunological--mutational analyses of **hCG** beta expressed via the transmembrane region of a MHC molecule made it possible to more precisely localize epitopes on **hCG**-derived molecules. Although the entire surface of **hCG** has to be regarded as potentially immunogenic there seems to be hot spots where epitopes are clustered in antigenic domains.. . . possibly centered around the knot itself. Ultimate answers on epitope localizations will be given by the crystal structure determination of **hCG** complexed with different Fabs.

L7 ANSWER 37 OF 40 MEDLINE
ACCESSION NUMBER: 92208566 MEDLINE
DOCUMENT NUMBER: 92208566 PubMed ID: 2980346

TITLE: Measuring human **chorionic gonadotropin** for detection of **early pregnancy loss**.
AUTHOR: Canfield R E; O'Connor J F; Wilcox A J
CORPORATE SOURCE: Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032.
CONTRACT NUMBER: HD 15454 (NICHD)
NO-1-ES-4-5054 (NIEHS)
RR 00645 (NCRR)
SOURCE: REPRODUCTIVE TOXICOLOGY, (1988) 2 (3-4) 199-203. Ref: 44
Journal code: BE4; 8803591. ISSN: 0890-6238.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920515
Last Updated on STN: 19990129
Entered Medline: 19920507

AB Elucidation of the primary molecular structure of **hCG**, coupled with monoclonal antibody technology, has permitted the construction of a partial map of **hCG** surfaces. Based on this information, two-site immunoradiometric assays have been developed which permit the measurement of intact **hCG** and its subunit molecular forms with unprecedented sensitivity and specificity. These assays have been employed in a determination of the incidence of **early pregnancy loss** in a normal population with the finding that 22% of all conceptions producing measurable **hCG** terminate before becoming clinically evident.

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L7 ANSWER 40 OF 40 MEDLINE DUPLICATE 26
ACCESSION NUMBER: 85285711 MEDLINE
DOCUMENT NUMBER: 85285711 PubMed ID: 4029425
TITLE: Measuring early pregnancy loss: laboratory and field methods.
AUTHOR: Wilcox A J; Weinberg C R; Wehmann R E; Armstrong E G; Canfield R E; Nisula B C
CONTRACT NUMBER: HD-15455 (NICHD)
RR-00645 (NCRR)
SOURCE: FERTILITY AND STERILITY, (1985 Sep) 44 (3) 366-74.
Journal code: EVF; 0372772. ISSN: 0015-0282.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198510
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19990129
Entered Medline: 19851011

AB We intensively studied 30 women attempting pregnancy in order to lay

groundwork for larger studies of **early pregnancy loss**. These women collected first morning urine specimens for up to 6 months after discontinuing use of birth control. Urine specimens were successfully collected for 98% of the woman-days in the study. Three assays for human **chorionic gonadotropin** (**hCG**) were performed on each urine specimen. An immunoradiometric assay (IRMA) specific to the carboxyterminal peptide of the **hCG** beta-chain proved to be more sensitive and more specific than two radioimmunoassays (RIAs). Using the IRMA, we found four cases in which **hCG** rose and fell over successive days, consistent with **early pregnancy loss**. For three of these four cases, the level of **hCG** was too low to be detectable with the RIAs. Among the control group of five women with tubal ligations, there was no detectable **hCG** above threshold with the IRMA. Thus, the enhanced sensitivity and specificity of the IRMA allows very early pregnancy losses to be identified that would otherwise be undetectable. Furthermore, its effectiveness with small quantities of first morning urine makes the IRMA a useful tool for epidemiologic studies.

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L3 ANSWER 1 OF 35 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:291336 BIOSIS
DOCUMENT NUMBER: PREV200100291336
TITLE: Isolation and partial characterization of early pregnancy factor (EPF.
AUTHOR(S): Haq, Afrozul (1); Al-Hussein, Khalid; Jaroudi, Kamal; Hollanders, J.; Shabani, Mohammad (1); Dawood Al-Waili, Noori S. (1)
CORPORATE SOURCE: (1) Dubai Specialized Medical Center and Research Labs, Dubai United Arab Emirates
SOURCE: FASEB Journal, (March 7, 2001) Vol. 15, No. 4, pp. A370. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001 Orlando, Florida, USA March 31-April 04, 2001
ISSN: 0892-6638.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Early pregnancy factor** (**EPF**), an immunosuppressive substance, which appears in pregnant women sera 48 hr after fertilization is a pregnancy-associated protein. We isolated **EPF** from sera of pregnant women positive for b-**hCG** by using DEAE-Sephadex A50 ion exchange chromatography. Complete fractionation was achieved in five peaks with increasing salt concentration (0 to 1M NaCl). Among all these peaks, peak 5 gave a maximum RIT (50%) versus 10% with non-pregnant sera in rosette inhibition assays. Proteins of peak 5 were also used at various concentrations and temperatures in RIT and SDS-PAGE. The other peaks (peaks 1,2,3,4) showed no or very little **EPF** activity. In mixed lymphocyte reaction (MLR) peak 5 showed an immunosuppressive effect. This suppressive effect was monitored as relative response to control e.g. (-1%) for peak 1, (6%) for peak 2, (10%) for peak 3, (8%) for peak 4 and (38%) for peak 5 respectively. There were two protein bands detected distinctively different from proteins of other peaks as analyzed by SDS-PAGE (12.5%). HPLC was performed for peak 5 and we purified and characterized a protein with 37 kDa as the possible candidate for **EPF**. Isolation and purification of **EPF** offers a basis for very early pregnancy diagnosis which has an important clinical applications as for women who conceive unexpectedly and could not become pregnant due risk factors.

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L3 ANSWER 9 OF 35 MEDLINE
ACCESSION NUMBER: 94369036 MEDLINE
DOCUMENT NUMBER: 94369036 PubMed ID: 8086632
TITLE: Isolation and characterization of early pregnancy factor.
AUTHOR: Zuo X; Su B; Wei D
CORPORATE SOURCE: Anhui Medical University, Hefei.
SOURCE: CHINESE MEDICAL SCIENCES JOURNAL, (1994 Mar) 9 (1) 34-7.
Journal code: A8E; 9112559. ISSN: 1001-9294.
PUB. COUNTRY: China
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941031
Last Updated on STN: 19941031
Entered Medline: 19941018

AB **Early pregnancy factor** (**EPF**) was purified from the pooled sera of 210 pregnant women at 3-8 weeks of gestation. Sera from healthy nonpregnant women were used as control. The samples (G-II, G-III and G-IV) obtained from pregnant women had **EPF** activity but no **HCG** activity. Polyacrylamide gel electrophoresis showed that the major bands in pregnant G-III and G-IV were at similar positions in tube gels. The results of SDS-PAGE showed 3 bands in pregnant G-IV: 57.0 kD, 38.0 kD and 19.0 kD. The basic active form of **EPF** may be a small peptide of 19.0 kD. The isoelectric points of pregnant G-IV were 6.45 and 8.20.

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L7 ANSWER 5 OF 40 SCISEARCH COPYRIGHT 2002 ISI (R) DUPLICATE 1
ACCESSION NUMBER: 2002:102282 SCISEARCH
THE GENUINE ARTICLE: 505PK
TITLE: Very low maternal serum PAPP-A and low free beta **hCG** are associated with **early pregnancy loss**
AUTHOR: Santolaya-Forgas J (Reprint); Hopkins R C; Sifuentes G; Castracane V D
CORPORATE SOURCE: Texas Tech Univ, Amarillo, TX USA
COUNTRY OF AUTHOR: USA
SOURCE: AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, (DEC 2001) Vol. 185, No. 6, Supp. [S], pp. S224-S224. MA 524.
Publisher: MOSBY, INC, 11830 WESTLINE INDUSTRIAL DR, ST LOUIS, MO 63146-3318 USA.
ISSN: 0002-9378.
DOCUMENT TYPE: Conference; Journal
LANGUAGE: English
REFERENCE COUNT: 0
TI Very low maternal serum PAPP-A and low free beta **hCG** are associated with **early pregnancy loss**

L7 ANSWER 7 OF 40 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2000:577575 SCISEARCH
THE GENUINE ARTICLE: 321MZ
TITLE: The determination of **early pregnancy loss** using measurement of human **chorionic gonadotropin** (**HCG**)-related forms with a modified urine specimen collection protocol.
AUTHOR: McChesney R (Reprint); Marcus M; OConnor J; Golden A; Landrigan P
CORPORATE SOURCE: COLUMBIA UNIV, BARNARD COLL, NEW YORK, NY 10027; COLUMBIA UNIV, IRVING CTR CLIN RES, NEW YORK, NY 10027
COUNTRY OF AUTHOR: USA
SOURCE: AMERICAN JOURNAL OF EPIDEMIOLOGY, (1 JUN 2000) Vol. 151, No. 11, Supp. [S], pp. 193-193.
Publisher: OXFORD UNIV PRESS INC, JOURNALS DEPT, 2001 EVANS RD, CARY, NC 27513.
ISSN: 0002-9262.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 0
TI The determination of **early pregnancy loss** using measurement of human **chorionic gonadotropin** (**HCG**)-related forms with a modified urine specimen collection protocol.

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WEST

Generate Collection

L2: Entry 9 of 9 File: JPAB Oct 23, 1992

PUB-NO: JP404300896A
DOCUMENT-IDENTIFIER: JP 04300896 A
TITLE: HUMAN-DERIVED EARLY PREGNANCY FACTOR AND SEPARATION AND PURIFICATION THEREOF

PUBN-DATE: October 23, 1992

INVENTOR-INFORMATION:
NAME COUNTRY
SUEOKA, HIROSHI
MURAKAMI, HIROSHI
BABA, ATSUSHI
KUSAMA, TAKESHI
MAKABE, OSAMU

ASSIGNEE-INFORMATION:
NAME COUNTRY
SUEOKA HIROSHI
MEIJI SEIKA KAISHA LTD

APPL-NO: JP03089162
APPL-DATE: March 29, 1991

INT-CL (IPC): C07K 15/06; A61K 37/02; C07K 3/20; G01N 33/53; A61K 49/00; G01N 33/50

ABSTRACT:

PURPOSE: To separate and purify the subject human early pregnancy factor capable of application to remarkably early pregnancy (fertilization) diagnosis, etc., from a crude raw powder of human villous gonadotropin.

CONSTITUTION: An hCG crude raw powder partly purified from human pregnancy urine is used as the starting raw material and fractionated by gel filtration, salting out, dialysis and chromatography to obtain a fraction having an EPF activity. EPF can be separated and purified by the above-mentioned process and the obtained hEPF has 2400-30000 molecular weight (measured by SDS polyacrylamide gel electrophoresis method) and the N-terminal amino acid sequence thereof is X-Ser-X-Gln-Asp-X-Ala-Pro-Val-Gly-X-Ser-Mer-Tyr-Ala-(X shows Cys or one of 20 kinds of natural amino acids).

WEST

Generate Collection Print

L8: Entry 1 of 11 File: USPT May 8, 2001

DOCUMENT-IDENTIFIER: US 6228660 B1
TITLE: Capillary immunoassay and device therefor comprising mobilizable particulate labelled reagents

Brief Summary Paragraph Right (18):
An important embodiment of the invention is a pregnancy testing device comprising a hollow elongated casing containing a dry porous nitrocellulose carrier which communicates indirectly with the exterior of the casing via a bibulous urine receiving member which protrudes from the casing and which can act as a reservoir from which urine is released into the porous carrier, the carrier containing in a first zone a highly-specific anti-hCG antibody bearing a coloured "direct" label, the labelled antibody being freely mobile within the porous carrier when in the moist state, and in a second zone spatially distinct from the first zone an highly-specific unlabelled anti-hCG antibody which is permanently immobilised on the carrier material and is therefore not mobile in the moist state, the labelled and unlabelled antibodies having specificities for different hCG epitopes, the two zones being arranged such that a urine sample applied to the porous carrier can permeate via the first zone into the second zone, and the casing being constructed of opaque or translucent material incorporating at least one aperture through which the analytical result may be observed, together with a removable and replaceable cover for the protruding bibulous urine receiving member. A fertile period prediction device, essentially as just defined except that the analyte is LH, is an important alternative.

Detailed Description Paragraph Right (21):
For example, if the test is used to determine the presence of hCG in urine during the course of a pregnancy test, the circular control zone 210 can contain immobilised HCG which will bind a labelled antibody which is carried upwards from zone 208 by the migrating liquid sample. The same labelled antibody can engage in a `sandwich` reaction with hCG in the sample and be bound in the first circular zone 209 by another specific anti-hCG antibody which has been immobilised therein. Alternatively, if desired, the "control" zone can be designed merely to convey an unrelated signal to the user that the device has worked. For example, the second circular zone can be loaded with an antibody that will bind to the labelled antibody from zone 208, e.g. an "anti-mouse" antibody if the labelled antibody is one that has been derived using a murine hybridoma, to confirm that the sample has permeated the test strip.

Detailed Description Paragraph Right (68):
A rectangular sheet of Schleicher and Schuell backed 8.mu. nitrocellulose measuring 25 cm in length and 20 cm in width may have a reaction zone formed upon it by applying a line of material about 1 mm wide at 5 cm intervals along its length and extending throughout its 20 cm width. The material can, for example, be a suitably selected antibody preparation such as anti-beta (human chorionic gonadotropin) of affinity Ka at 10.sup.9, prepared in phosphate buffered saline pH 7.4 at 2 milligram/ml, suitable for immunoassay of human chorionic gonadotrophin using a second (labelled) anti-hCG antibody in a sandwich format. This solution can be deposited by means of a microprocessor-controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 2 mm diameter. When the applied material has been allowed to dry for 1 hour at room temperature, excess binding sites on the nitrocellulose are blocked with an inert compound such as polyvinyl alcohol (1% w/v in 20 mM Tris pH 7.4) for 30 minutes at room temperature, and sheets are thoroughly rinsed with distilled water prior to drying for 30 minutes

at 30.degree. C.

CLAIMS:

11. A pregnancy testing device for detecting human chorionic gonadotrophin (hCG) suspected of being present in a urine sample and requiring solely the application thereto of said urine sample to enable a test to be performed and a test result to be provided, said device comprising:

a hollow casing constructed of moisture impervious solid material;

a test strip, comprising a dry porous carrier disposed in said casing, said dry porous carrier having a detection zone and a control zone; and

labeled reagent comprising an anti-hCG antibody bearing a particulate direct label capable of specifically binding with said hCG to form a first complex of said labeled anti-hCG antibody and said hCG, said labeled hCG antibody reagent being at least one of disposed on and contained in said test strip in a dry state prior to use,

said casing comprising a sample application aperture through which urine can be applied directly or indirectly to said carrier, said labeled hCG antibody being located upstream from said detection zone prior to use and released into mobile form by application of said urine sample,

wherein said carrier comprises, immobilized in said detection zone, means for binding said first complex, and wherein migration of said applied urine sample through said dry porous carrier conveys by capillarity said labeled hCG antibody and said hCG to said detection zone of said dry porous carrier, whereat said binding means binds said first complex thereby to form a second complex, thereby to indicate the presence of said hCG in said urine sample,

said casing comprising a test result observation aperture remote from said sample application aperture, said detection zone at least being visible through said test result observation aperture, said sample application aperture and said test result observation aperture being spaced apart and disposed so that urine can be applied to said sample application aperture to initiate said test but said test strip is substantially shielded from accidental initial application of urine directly to a portion of said test strip downstream from said location of said labeled hCG antibody in the dry state,

wherein said control zone comprises a material for indicating that said urine sample has been conveyed thereto by capillarity along said carrier irrespective of a presence or absence of hCG in said urine sample.

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L2: Entry 1 of 9 File: USPT Jan 9, 2001

DOCUMENT-IDENTIFIER: US 6172198 B1
TITLE: PAPP-A, its immunodetection and uses

Brief Summary Paragraph Right (3):
A number of placental proteins have now been isolated and at least partially characterised. These include--human chorionic gonadotropin (hCG), pregnancy-specific .beta..sub.1 --glycoprotein (SP1), placental protein 5 (PP5), early pregnancy factor (EPF), and pregnancy-associated plasma protein-A (PAPP-A).sup.1.

Brief Summary Paragraph Right (4):
These proteins are detectable, in maternal blood, at various stages during pregnancy. For example, EPF activity is detectable within 24 hours after conception. HCG is measurable just after implantation, at about 9 to 11 days post-ovulation, SP1 is detectable from 18 to 23 days post-ovulation. In singleton pregnancies, PAPP-A can be detected approximately 28-32 days post-ovulation.sup.2.

Brief Summary Paragraph Right (5):
Placental proteins are also detectable for varying periods during pregnancy. For example, EPF is detectable at least for the first half of pregnancy, whereafter activity declines until it is totally absent during the third trimester in some women. HCG levels rise rapidly to peak at about 8 to 12 weeks gestation. The levels of SP1 rise exponentially with peak concentrations being reached at term pregnancy. Like SP1, PAPP-A concentrations also rise exponentially in the first trimester of pregnancy to peak at term.sup.2.

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L2: Entry 3 of 9 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981198 A
TITLE: Preimplantation factor

Brief Summary Paragraph Right (8):
Early pregnancy factor (EPF) binds to lymphocytes and decreases rosette formation in the sheep red blood cell inhibition assay (RIT) (3). By contrast, in our assay PIF enhances lymphocyte binding to platelets demonstrating the ability of PIF to cause enhanced platelet-lymphocyte interactions. Whatever the mechanisms of action, PIF can be measured in our bioassay by virtue of its enhancement of interaction between platelets and lymphocytes. This phenomenon is pregnancy specific, and is not the result of actions of pregnancy hormones such as progesterone or human chorionic gonadotropin (hCG).

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L6: Entry 5 of 5 File: DWPI Oct 23, 1992

DERWENT-ACC-NO: 1992-403416
DERWENT-WEEK: 199249

TITLE: Human derived early pregnancy factor - increases rosette inhibition reaction, has mol weight 24000 to 30000, has specific aminoacid sequence

Basic Abstract Text:
Human derived early pregnancy factor increases rosette inhibition reaction. Molecular. is 24,000-30,000 (SDS polyacrylamide gel electrophore sis). Isoelectric point is 3.5-3.75. Aminoacid sequence of N-terminal is formulated as X-Ser-X-Gln-X-Asp-X-Ala- Pro-Val-Gly-X-Ser -Met-Tyr-Ala. (X is Cys or one aminoacid in 20 kinds of aminoacid existing naturally). Human derived early pregnancy factor is sepd. and purified comprising deriving crude powder of human chorionic gonadotropin from urine of human normal gravida suspending in buffer, and sonificating. Supernatant obtd. by centrifugation is fractionated by Sephalose Cl-6B column gel filtration, and active fraction lately eluting than that of human chorionic gonadotropin is collected. The fraction is salted out with 70% satd. (NH4)2SO4. After ppte. is dialysed against Tris buffer, it is treated by Q Sephalose Fast Flow chromatography and NaCl linear gradient elution. Active fraction is dialysed against (NH4)2SO4 contg. buffer, treated by phenyl Sephalose CL-4B column. Eluting active fraction is collected and dialysed against H2O and lyophilised to prepare partially purified prod. This is dialysed against H2O once more, and purified by reverse phase HPLC.

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L9: Entry 4 of 14 File: USPT Sep 12, 2000

DOCUMENT-IDENTIFIER: US 6117421 A
TITLE: Method for promoting cell growth and immunosuppression using chaperonin

Brief Summary Paragraph Right (6):
EPF was first described as a pregnancy associated substance (Morton et al., 1976, Proc. R. Soc. B. 193 413-419) and its discovery created considerable interest as it enabled the detection of a potential pregnancy within 6-24 hours of fertilisation. Initially EPF was assigned a role as an immuno-suppressant by virtue of its ability to release suppressor factors from lymphocytes (Rolfe et al., 1988, Clin. exp. Immunol. 73 219-225). These suppressor factors depress the delayed type hypersensitivity reaction in mice and therefore might suppress a possible maternal immune response against the antigenically alien fetus. More recent studies have shown that production of EPF is not confined to pregnancy. It is a product of primary and neoplastic cell proliferation and under these conditions acts as a growth factor (Quinn et al., 1990, Clin. exp. Immunol. 80 100-108; Cancer Immunol. Immunother, 1992, 34 265-271). EPF is also a product of platelet activation and it is proposed therefore that it may play a part in wound healing and skin repair (Cavanagh et al., 1991, Journal Reproduction and Fertility 93, 355-365).

Brief Summary Paragraph Right (7):
To date, the rosette inhibition test remains the only means of detecting EPF in complex biological mixtures (Morton et al., 1976, Proc R Soc B 413-419). This assay is dependent on the original finding of Bach and Antoine, 1968, Nature (Lond) 217 658-659 that an immunosuppressive anti-lymphocyte serum (ALS) can inhibit spontaneous rosette formation in vitro between lymphocytes and heterologous red blood cells. A modification of the assay was introduced to detect EPF after it was demonstrated that lymphocytes, preincubated in EPF, give a significantly higher rosette inhibition titre (RIT) with an ALS than do lymphocytes from the same donor without EPF as described in the 1976 reference above. This test has been described in detail in the above 1976 reference as well as in Morton et al., 1987, in "In Current Topics in Developmental Biology" Vol 23 73-92, Academic Press, San Diego, but briefly it involves a cascade of events with EPF binding to lymphocytes and sequentially inducing the release of suppressor factors (Rolfe et al., 1988, Clin. exp. Immunol. 73 219-225); (Rolfe et al., 1989, Immunol. Cell Biol. 67 205-208).

Brief Summary Paragraph Right (8):
In Athanasas-Platsis et al., 1989, Journal Reproduction and Fertility 87 495-502 and Athanasas-Platsis et al., 1991, Journal Reproduction and Fertility 92 443-451, there is described the production of monoclonal and polyclonal antibodies to EPF and passive immunization of pregnant mice with these antibodies which causes loss of embryonic viability. These studies established that EPF is necessary for the successful establishment of pregnancy.

Brief Summary Paragraph Right (9):
In Quinn et al., 1990, Clin. exp. Immunol. 80 100-108, it is proposed that EPF is a growth regulated product of cultured tumour and transformed cells. These cells are also dependent upon EPF for continued growth i.e. EPF acts in an autocrine mode.

Brief Summary Paragraph Right (10):
It has been established that EPF plays a role in promoting tumour growth since the growth of tumour cells can be significantly retarded by anti-EPF mAbs. In addition this reference suggests that hybridomas producing high affinity anti-EPF antibodies may be inherently unstable.

Brief Summary Paragraph Right (11):
In Quinn et al., 1992, Cancer Immunol. Immunother, 34 265-271, there is also described the effect of monoclonal antibodies (mAbs) to EPF on the in vivo growth of transplantable murine tumours. The main thrust of this reference is that neutralisation of EPF retards tumour growth in vivo.

Brief Summary Paragraph Right (12):
It is clear from the above Quinn et al. 1992 reference that when cancer is in the very early stage of growth, neutralisation of EPF by anti-EPF mAb will prevent its development. However, once the cancer becomes established, treatment with these mAbs will retard but not entirely destroy the tumour.

Brief Summary Paragraph Right (13):
Other references in regard to the role of EPF in tumour growth include Quinn, 1991, Immunol. Cell Biol. 69 1-6 and Quinn, K. A. in a PhD thesis entitled "Early pregnancy factor: a novel factor involved in cell proliferation.sup.11 from the University of Queensland in Australia in 1991.

Brief Summary Paragraph Right (14):
EPF is reviewed in detail by Morton et al., 1992, Early Pregnancy Factor, Seminars in Reproductive Endocrinology 10 72-82. The site and regulation of EPF production is described, followed by the purification of EPF from platelets and the relationship of the purified product to EPF derived from other sources. This review also discusses certain aspects of the bioassay for EPF (i.e. the rosette inhibition test) including monitoring purification procedures and investigating sources of production. The biological activity of EPF is discussed and possible clinical applications of EPF and its antagonists are described.

Brief Summary Paragraph Right (15):
Morton et al., 1992, Reprod. Fertil Dev. 4 411-422 reviews previous publications describing the immuno suppressive and growth factor properties of EPF. The role of EPF in maintaining the pre-embryo is also discussed in this reference.

Brief Summary Paragraph Right (16):
Both of the abovementioned references, which are essentially review articles, describe the preparation of purified EPF for blood platelets which included the initial sequential steps of heat extraction of the platelets, cation exchange chromatography on SP-SEPHADEX, crosslinked dextran beads C-25, affinity chromatography on Heparin-SEPHAROSE, crosslinked agarose beads CL-6B and Concanavalin-A-Sepharose 4B. The final purification of EPF was achieved by high performance hydrophobic interaction chromatography, followed by three reversed phase (RP)-HPLC steps. After the final RP-HPLC step, EPF was isolated as single UV absorbing peak coincident with biological activity, well separated from a number of minor contaminants. The biological and radioactivity of an iodinated sample of this material eluted with identical retention time when fractionated under the same conditions. When analysed by SDS-PAGE and visualised by autoradiography, the iodinated material ran as a single band of approximate Mr 10,000, again coincident with biological activity. The approximate yield of EPF by this method was 5 .mu.g per 100 platelet units.

Brief Summary Paragraph Right (17):
This demonstrates that it was necessary to use this complex purification procedure to obtain only a small amount of native EPF and thus this method could not be used on a commercial scale. In this regard, the only practical sources known for obtaining native EPF at this time were platelets and regenerating liver.

Brief Summary Paragraph Right (18):
Surprisingly, in accordance with the present invention, the final fractionated EPF when subjected to sequencing as more fully described hereinafter found that the structure of native EPF corresponded to chaperonin 10 which could not have been predicted from the aforementioned prior art.

Brief Summary Paragraph Right (19):
This unexpected discovery as will be apparent from the disclosure hereinafter has

now been reduced to practice in that recombinant chaperonin 10 has been found to have all the biological activity previously associated with EPF and thus EPF can now be produced commercially which was not the case previously using suitable techniques for producing recombinant cpn10. It will also be apparent that EPF can now be produced synthetically.

Brief Summary Paragraph Right (20):
In one aspect, the invention resides in the discovery that cpn10 is EPF and has the hitherto unknown or unsuspected properties demonstrated by EPF. The unknown or unsuspected properties of cpn10 include extracellular activities such as the ability to act as a growth factor and an immunosuppressive factor. In another aspect the invention provides one or more methods for using cpn10 to exploit the unknown or unsuspected properties of cpn10. The one or more methods includes a method of using cpn10 to promote growth and a method of using cpn10 to suppress immunological activity.

Brief Summary Paragraph Left (1):
Early Pregnancy Factor (EPF)

Drawing Description Paragraph Right (2):
Purification of EPF. Heat extracted human platelets (100 units) were fractionated on SP-SEPHADEX and Heparin SEPHAROSE, then applied to a TSK-Phenyl 5PW column and eluted with a reverse salt gradient. Fractions were tested in the rosette inhibition test (based on EPF's capacity to augment the rosette inhibiting activity of an immunosuppressive antilymphocyte serum).

Drawing Description Paragraph Right (10):
Interaction of immobilised monoclonal anti-EPF antibody 5/341 with active fractions from (d) and equivalent fractions from human pregnancy serum, 6 d gestation (10 ml); human pregnancy urine, up to 1 month gestation (10 liter); medium conditioned by oestrous mouse ovaries (100) stimulated with prolactin and mouse embryo-conditioned medium (ovary CM); serum free medium conditioned by the bovine kidney cell line MDBK (MDBK-CM; ATCC CCL 22, 10 liter); rat serum obtained 24 h post-partial hepatectomy (post-pH, 10 ml); rat liver obtained 24 h post-pH (40 g); all fractionated as in (a) to (d). Anti-EPF bound and unbound fractions were tested in the rosette inhibition test, specificity was demonstrated by comparison with a parallel experiment using irrelevant antibody in which activity was not bound.

Drawing Description Paragraph Right (12):
Analysis of EPF purified from 300 units human platelets as in FIG. 1A. Determination of monomeric size. Iodinated EPF was fractionated by SDS-PAGE,.sup.29 the gel sliced (2 mm wide slices) and the distribution of radioactivity and biological activity compared. (Inset) Direct Coomassie Blue staining of the same preparation.

Drawing Description Paragraph Right (14):
Ion-spray mass spectrum of EPF, displayed as multiply protonated molecular ions.

Drawing Description Paragraph Right (18):
Amino-acid sequence (single letter code) of peptides derived from human EPF, compared with rat cpn10 (underlined). EPF was digested with endoproteinase lys C and endoproteinase glu C, the resultant peptides separated by RP-HPLC and sequenced. The sequence of individual fragments is shown; all except 74-101 were derived from the lys digest.

Drawing Description Paragraph Right (20):
Peak fractions in the excluded volume of a TSK G3000SW gel permeation column, following application of a cpn60-EPF mixture +Mg.sup.2+ ATP, were analysed by SDS-PAGE (Schagger et al., 1987) and stained with silver (Morrissey, 1981). Left lane, +ATP; right lane -ATP. (Cpn60 is a decatetramer, M, 840 000; column exclusion limit >300 000. Higher M.sub.r bands on SDS gel are oligomeric forms of groEL).

Detailed Description Paragraph Right (14):
EPF has been purified from various sources as discussed in Cavanagh & Morton, 1994, Eur. J. Biochem. 222 551-560; Quinn et al., 1994, Hepatology 20 No 5 1294-1302.

Detailed Description Paragraph Right (15):
In all instances, biological activity followed the same pattern throughout the complex purification scheme described above for human platelets. Furthermore the final active fraction from all sources was bound specifically by an immobilised monoclonal anti-EPF and could be recovered virtually quantitatively (see FIG. 1e).

Detailed Description Paragraph Right (17):
Human platelet-derived EPF, being most abundant, has been studied in some detail. On SDS-PAGE, it ran as a single band of Mr approx, 8.500, coincident with biological activity (see FIG. 2a); EPF from regenerating rat liver exhibited identical behaviour. Mass spectometry of the platelet material provided an accurate and precise determination of molecular mass 10 843.5.+-.2 Da, along with definitive evidence of the high degree of homogeneity of the preparation (see FIG. 2b). Following attempts at Edman degradation, which indicated that the molecule is N-blocked, proteolytic cleavage of approx. 4 nmol EPF was undertaken. Resultant peptide fragments were separated by reversed-phase HPLC and subjected to sequencing by Edman degradation. Three areas of sequence containing 12 (fragment 1), 27 (fragment 2) and 33 (fragment 3) residues were found to correspond with residues 7 to 18-27-53 and 69 -101 (the C-terminus) in rat mitochondrial cpn10. In fragment 2, residue 52 was different (S in cpn10, G in rat cpn10, this change alone could account for human cpn10 being 30 Da larger than rat cpn10). All other residues were identical, consistent with the highly conserved nature of chaperonins (see FIG. 2c).

Detailed Description Paragraph Right (18):
Since confirming sequence identity between EPF and cpn10 several studies of functional relationship have been performed, using rat mitochondrial cpn10 E. coli cpn10 (known as groES) and E. coli cpn60 (groEL). First it has been demonstrated that cpn10 can act as EPF. Rat cpn10 was tested in the EPF bioassay and found to be positive over the range of dilutions expected; this activity could be neutralised by monoclonal antibodies to EPF (see TABLE 1). Interestingly, E. coli cpn10, which is .about. 40% homologous with rat cpn10, exhibited no activity in the bioassay (see TABLE 1): this is consistent with the observation that E. coli conditioned medium is not active in the EPF bioassay, while medium conditioned by all mammalian cell lines tested, as well as by yeast cells is active. Cpn60 was inactive in the bioassay and had no effect upon the activity of EPF. It was then shown that EPF can act as cpn10. EPF was mixed with cpn60 , in the presence or absence of ATP, and the mixture fractionated on a TSK G3000SW gel permeation column: resultant fractions were analysed by SDS-PAGE. Cpn60 is a decatetramer and elutes in the excluded volume of this column (exclusion limit 300 000). In the presence of ATP, but not in its absence, EPF also appears in this fraction, demonstrating formation of a stable complex with cpn60. This fraction was active in the EPF bioassay but the equivalent fraction from the experiment without ATP (where EPF did not associate with cpn60) was not (see FIG. 3a). Thus EPF and cpn10 activity reside in the same molecule.

Detailed Description Paragraph Right (19):
These investigations provide unequivocal evidence that platelet-derived EPF is a structural and functional homologue of cpn10the relationship between cpn10 and activity in the rosette inhibition test was then examined (FIG. 3b). In the presence, but not in the absence of ATP, immobilised cpn60 could remove all activity from the archetypal source material, pregnancy serum and activity could be recovered by removing ATP from the immobilised complex. As with the experiment described in FIG. 3a, this requirement for ATP demonstrates the specificity of the interaction between cpn60 and the active moiety; cpn10 is thus the molecular entity initiating response in the EPF bioassay.

Detailed Description Paragraph Right (20):
Identification of EPF as a cpn10 has been a major step forward in research on this subject and helps to explain many of the findings that have been made to date. Criticism has been raised against claims that EPF production occurs in such a wide variety of biological situations e.g. pre- and post-implantation pregnancy, primary and tumour cell proliferation and platelet activation. In its role as a hsp (heat stress protein) following the advent of the present invention, these are all conditions in which the rapid onset of EPF production would now be expected. Functions of hsp's that are vital to the survival of cells are intracellular as

shown in the Linquist et al. reference above. In contrast, the activity of EPF described to date is extracellular; for example, it appears in serum of mice within 4 to 6 hours after mating as discussed in Morton et al., 1987, Current Topics in Development Biology, Vol 23 73-92 and 4 to 8 hours after partial hepatectomy in rats as shown in the Quinn PhD thesis (1991), available from the Biological Sciences Library, University of Queensland Australia, catalogued under both author and title. We have shown that EPF can act in an autocrine mode as discussed in the Quinn et al., 1990 reference referred to above or exocrine mode as discussed in the Rolfe et al. 1988 referred to above; these are not roles previously described for hsp's.

Detailed Description Paragraph Right (21):
It will also be appreciated that since the structure of EPF is now known, it can be produced in commercial quantities by any suitable technique of recombinant DNA technology.

Detailed Description Paragraph Right (77):
The data referred to herein also provides clear support for the use of cpn10 in treatment of inflammatory conditions including inflammatory bowel disease and infectious disease. Such data as described herein includes references drawn from the immunosuppressive effect of cpn10 in the rat EAE and skin graft models. This is also supported by Rolfe et al., 1983, Clin. exp. Immunol. 51 45-52 and Nature 278 No. 5705 649-651 showing that EPF can reduce delayed type hypersensitivity in mice.

Detailed Description Paragraph Right (78):
The use of cpn10 in treatment of allergic disease including allergic rhinitis, asthma, atopic dermatitis, acute urticaria and drug hypersensitivity is also fully supported by the immunosuppressive effect of cpn10 in the rat EAE and skin graft models. This conclusion can also be drawn from Rolfe et al., 1983, Clin. exp. Immunol. 51 45-52 and Noonan et al., 1979, Nature 278 No. 5705 649-651 showing effect of EPF in reducing delayed type hypersensitivity in mice.

Detailed Description Paragraph Left (11):
Purification of EPF from other sources

Detailed Description Paragraph Left (22):
Sensitivity of the Rosette Inhibition Test, the EPF Bioassay

Detailed Description Paragraph Center (3):
(a) Purification of Human EPF from Human Blood Platelets (FIG. 1a, 1b, 1c, 1d)

Detailed Description Paragraph Type 1 (12):
B. The active agents purified from all of these materials are from several to many orders of magnitude more potent than virtually all of the substances previously reported to be EPF. This confirms our surmise, based on detailed analysis of the EPF bioassay as discussed above, that activity associated with most putative EPF preparations must reflect the presence of a very minor contaminant.

Detailed Description Paragraph Type 1 (13):
C. The only source materials providing sufficient EPF to study at the protein (as opposed to activity) level were platelets and regenerating liver, yielding, respectively, an average of 15 .mu.g per 100 units (equivalent to .about.50 liter blood) and 5 .mu.g per 40 g tissue (liver remnant from 6 rats). It is immediately apparent that far more EPF is present within the cell than appears in the extracellular space; nevertheless, accumulated knowledge of the biology of EPF (reviewed recently in the abovementioned Morton et al. 1992 reference) indicates that this extracellular appearance is not fortuitous.

Detailed Description Paragraph Table (1):
Abbreviations
ANGIS Australian National Genomic Information Service bp base pair BSA bovine serum albumin cDNA complementary DNA cpn 10 Chaperonin 10 DNA deoxyribonucleic acid E. coli Escherichia coli GSH glutathione (reduced form) GST glutathione-S-transferase LB Luria-Bertani Broth M Molar ORF open reading frame PCR polymerase chain reaction rEPF recombinant Early Pregnancy Factor RSP reverse sequencing primer SDS sodium dodecyl sulphate SDS-PAGE sodium dodecyl

sulphate-polyacrylamide gel electrophoresis Tris Tris(hydroxymethyl)aminomethane USP universal sequencing primer _____

Detailed Description Paragraph Table (2):
TABLE 1 _____ Limiting Dose (log reciprocal) Sample Untreated + 5/341 _____ Human platelet EPF 13 <2 (50 .mu.g/ml) Rat liver cpn 10 (50 13 <2 .mu.g/ml) E. coli cpn 10 (groES) NA NT (50 .mu.g/ml) _____

Other Reference Publication (2):
Rolfe et al., "Early Pregnancy Factor is an Immunosuppressant Contaminant of Commercial Preparations of HCG," Clin. Exp. Immunol. 51: 45-52, 1983.

Other Reference Publication (3):
Rolfe et al., "Identification of Two Suppressor Factors Induced by Early Pregnancy Factor," Clin. Exp. Immunol. 73:219-225, 1988.

Other Reference Publication (7):
Noonan et al., "Early Pregnancy Factor is Immunosuppressive," Nature 278: 649-651, 1979.

Other Reference Publication (8):
Cavanagh et al., "The Purification of Early-Pregnancy Factor to Homogeneity From Human Platelets and Identification As Chaperonin 10", Eur. J. Biochem., vol. 22:551-560, (1994).

Other Reference Publication (9):
Quinn et al., "Early Pregnancy Factor In Liver Regeneration After Partial Hepatectomy In Rats: Relationship With Chaperonin 10", Hepatology, vol. 20:1294-1302, (1994).

Other Reference Publication (10):
Quinn et al., "Monoclonal Antibodies To Early Pregnancy Factor Perturb Tumour Cell Growth", Clin. exp. Immunol., vol. 80:100-108, (1990).

Other Reference Publication (11):
Quinn et al., "Effect of Monoclonal Antibodies to Early Pregnancy Factor (EPF) On The Vivo Growth of Transplantable Murine Tumours", Cancer Immunol. Immunother, vol. 34:265-271, (1992).

Other Reference Publication (19):
Cavanagh, A.C. et al., "Relationship between early pregnancy factor, mouse embryo-conditioned medium and platelet-activating factor", Journal of Reproduction & Fertility, 93:355-365, 1991.

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L9: Entry 11 of 14 File: USPT Oct 31, 1989

DOCUMENT-IDENTIFIER: US 4877742 A
TITLE: Pregnancy test with EPF

Abstract Paragraph Left (1):
A method for the early detection of a pregnancy comprises determination of EPF in the blood or urine or in a biochemical derivative of the blood or urine of pregnant women, by measuring the influence of EPF on the production of electronically excited states or radicals, especially oxygen radicals, or their secondary products, in leukocyte or cell line preparations. The method can also be used to determine gonadal tumors.

Brief Summary Paragraph Right (1):
EPF (Early Pregnancy Factor) is a protein that during pregnancy occurs in the blood or urine or in biochemical derivatives of blood or urine of pregnant women. In addition to its presence during pregnancy, EPF also occurs in these fluids when gonadal tumors exist (testicular or ovarian tumors) [Aust. J. Biol. Sci. 37 (1984) 393-407]. [Fertility and Sterility 35 (1981) 397-402]

Brief Summary Paragraph Right (2):
EPF binds on lymphocytes and can be detected in the rosette inhibition test. The test is based on the fact that EPF intensifies the inhibition of the active rosette formation between lymphocytes and heterologous erythrocytes by antilymphocyte serum [Proc R. Soc. London Ser. B. 193 (1976) 413-149]. In the rosette inhibition test, EPF can be detected in the serum of pregnant women already within 24 hours after conception (union of ovum and sperm) and 2-3 days later also in the urine of these women. In the case of in vitro fertilization a successful embryo transfer is indicated by the occurrence of EPF about 3 days after the transfer (Ann. N.Y. Acad. Sci. 442:420-8, 1985).

Brief Summary Paragraph Right (3):
Therefore, with the aid of EPF diagnostics, a pregnancy can be detected at a very early moment (1-5 days after conception). However, the rosette inhibition test is labor-and time-consuming; moreover, it is subject to a high error rate. Even a positive pregnancy is often detected only after repeated tests. On the other hand, to rule out a pregnancy, several repetitions of the test are always necessary.

Brief Summary Paragraph Right (4):
Therefore there is a great interest in a simple and reliable process for early detection of a pregnancy based on the occurrence of EPF.

Brief Summary Paragraph Right (7):
It has now been found that EPF exerts a measurable influence on the formation of electronically excited states or radicals, especially oxygen radicals and their secondary products, in a leukocyte preparation or in cell lines. If an EPF-containing serum or EPF-containing urine concentrate is incubated with a leukocyte preparation or with a cell line preparation, and the cells are washed and suitably activated to cause the formation of electronically activated states or radicals, especially oxygen radicals, or their secondary products, then the formation of said states or radicals will be different in the presence of EPF than when EPF is absent, and that difference can be measured. In the case of oxygen radical formation, measured, for example, with luminescence, cytochrome C reduction or H.sub.2 O.sub.2 determination, a change of the radical flux is found in comparison with the controls not treated with EPF. In the case of the controls, the

leukocyte or cell line preparation is incubated with serum or urine concentrate or their biochemical derivatives of nonpregnant women. Generically, the electronically activated states refer to excited states of moieties in the preparations wherein electrons are in energy levels above their ground states.

Brief Summary Paragraph Right (8):
The invention therefore relates to a method for the early detection of a pregnancy, based on the measurement of EPF's existence by determining this influence of EPF on the formation of electronically activated states or radicals, especially oxygen radicals, or their secondary products, in a leukocyte preparation or in cell lines.

Brief Summary Paragraph Right (9):
The method according to the invention is also suitable for diagnosis of gonadal tumors in male and female humans, when EPF is detected in accordance with the foregoing and, in females, pregnancy is ruled out.

Brief Summary Paragraph Right (12):
Suitable preparations of the preferred embodiment of EPF test medium (A) and alternative embodiments (B) are described in the following.

Brief Summary Paragraph Right (15):
The foregoing preparation details and examples of suitable cells are exemplary only. Amounts, values, processing treatments, etc., can all be varied as long as the resultant preparation is capable of functioning in the method of this invention as described herein. For example, human leukocytes may be obtained from female donors, provided hat these are known not to be pregnant. It is possible that leukocytes from other species will respond to human EPF ("Pregnancy Proteins" (ed. Crudzinskas J., Teisner B. and Seppala M) Academic Press 391-405)

Brief Summary Paragraph Right (18):
Typically, the number of cells per test sample will be in the range of 10.sup.3 to 10.sup.7 when used in conjunction with the EPF-containing preparations discussed below.

Brief Summary Paragraph Right (19):
Suitable EPF sources include:

Brief Summary Paragraph Right (21):
Suitable biochemical derivatives include those conventional derivatives which retain the activity of EPF in the original fluid, e.g., extracts, fractions or concentrates of these fluids prepared by ultrafiltration, chromatographic separation, electrophoretic separation or lyophilization (J. Immunol. Methods 70 (1984) 1-11 and "Early Pregnancy Factors" (ed. F. Ellendorff & E. Koch) Perinatology Press, Ithaca, N.Y.).

Brief Summary Paragraph Right (24):
For detection of EPF activity, the mononuclear cells are stimulated for formation of electronically excited states or free radicals, especially oxygen radicals or their secondary products. This preferably takes place by addition of phorbol myristate acetate (PMA) in an end concentration of 20-30 ng/ml. Alternatively other dosages of PMA or other stimuli (other phorbol esters, ionophores, detergents, fluoride ions, activation by Fc- or complementary receptors) can be used.

Brief Summary Paragraph Right (33):
Using such measurement systems, EPF causes a measurable change in the formation of electronically excited states or radicals, especially oxygen radicals and their secondary products, when contrasted with the results where no EPF is present.

Brief Summary Paragraph Right (34):
In certain circumstances, other substances may interfere with the measurement of EPF (see Example in Table 2, Samples from Clinic H). It is likely that this problem can be circumvented by partial purification of the samples (J. Immunol. Methods 70 (1984) 1-11). Since, as discussed above, leukocytes or their derivatives can be stimulated by a variety of agents to produce radicals, definitive correlation with the presence of EPF is most readily demonstrated by comparison of serum with a

control serum taken from the same person before conception has occurred.

Brief Summary Paragraph Right (35):
Using the method of this invention, early pregnancies are detectable by observation of a statistically significant increase in EPF levels over the standard control values. Typically, levels increase by 25-400% in humans. Where EPF levels are increased in males or in females where no pregnancy is found, then the result indicates the presence of a gonadal tumor.

Brief Summary Paragraph Right (36):
For this invention, it is necessary to utilize a control sample. In the preferred mode, a sample from the same patients will be used where possible; thus, where in vitro fertilization or artificial insemination is involved or where a female plans natural conception in the near future, adequate samples can be taken prior to the planned events. In this case, the levels of electronic excited states or radical formation in the presence of the patient's own control fluid and the leukocyte or cell sample can be obtained and compared with the raised level in the presence of exactly the same components except for the generated EPF. Where this is not possible, e.g., where it might be expected that a male or female has a gonadal tumor, the EPF determination according to this invention can be carried out and compared with average control levels taken over a statistically relevant number of samples. These average values will be routinely determinable and, of course, will vary with the method used to generate the radical or other excited state. For instance, the average control value for the technique utilized in example (a) below is approximately 400-2000 cpm and in example (b) below it is approximately 100-300 cpm (cpm=counts per minute).

Detailed Description Paragraph Right (1):
The first blood sample was taken 4-10 days before embryo transfer; other blood samples were taken between 5 and 11 days after embryo transfer. The success of the transfer was evaluated by the usual signs of pregnancy (stopping of the period, positive HCG detection with radioimmunoassay).

Detailed Description Paragraph Right (11):
By daily treatment with HCG doses of 2-10 thousand IU, the differences between a positive and negative course of embryo transfer are blurred (Table 2, samples from Clinic H).

Detailed Description Paragraph Center (2):
Detection of EPF in sera on women after embryo transfer

Other Reference Publication (3):
Morton et al., "The Appearance and Characteristics of Early Pregnancy Factor in the Pig," J. Reprod. Fert., (1983), 69, 437-446.

Other Reference Publication (4):
Nancarrow et al., "The Early Pregnancy Factor of Sheep and Cattle," J. Reprod. Fert., Suppl. 30, (1981), 191-199.

Other Reference Publication (17):
Clarke, F. and S. Wilson, In Early Pregnancy Factors, F. Ellendorf and E. Koch, eds., Perinatology Press; pp. 165-177, (1985).

CLAIMS:

1. A method for early detection of a pregnancy in a female mammal, which does not have a gonadal tumor, comprising determining EPF by measuring its effect on the formation of electronically excited states or free radicals by mononuclear cells or a cell line in a test sample of the female mammal's blood or urine wherein said formation is caused by a treatment effective to form said states or radicals, and comparing said effect to the effect on said formation of a control sample comprising corresponding material from at least one nonpregnant female, which does not have a gonadal tumor, of the same mammalian species, wherein a statistically significant difference between the effect of the test sample compared to the effect of the control sample indicates pregnancy.

2. A method of claim 1 wherein the sample from the female tested for EPF is serum, plasma or urine.

3. A method of claim 1, wherein the sample of blood or urine from the female tested for EPF has been treated in a manner designed to concentrate or enrich the EPF present or to remove species which will interfere with said method.

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L9: Entry 12 of 14 File: JPAB Oct 23, 1992

DOCUMENT-IDENTIFIER: JP 04300896 A
TITLE: HUMAN-DERIVED EARLY PREGNANCY FACTOR AND SEPARATION AND PURIFICATION THEREOF

Abstract (1):
PURPOSE: To separate and purify the subject human early pregnancy factor capable of application to remarkably early pregnancy (fertilization) diagnosis, etc., from a crude raw powder of human villous gonadotropin.

Abstract (2):
CONSTITUTION: An hCG crude raw powder partly purified from human pregnancy urine is used as the starting raw material and fractionated by gel filtration, salting out, dialysis and chromatography to obtain a fraction having an EPF activity. EPF can be separated and purified by the above-mentioned process and the obtained hEPF has 2400-30000 molecular weight (measured by SDS polyacrylamide gel electrophoresis method) and the N-terminal amino acid sequence thereof is X-Ser-X-Gln-Asp-X-Ala-Pro-Val-Gly-X-Ser-Mer-Tyr-Ala-(X shows Cys or one of 20 kinds of natural amino acids).